PATENT
Docket No. 104914.127
(formerly 2844/53802)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appellants	:	Leiden *et al.*
Serial No.	:	09/473,830
Filing Date	:	December 28, 1999
For:	:	Efficient and Stable In Vivo Gene Transfer to Cardiomyocytes Using Recombinant Adeno-Associated Virus Vectors
Examiner	:	Chen, Shin-Lin
Art Unit	:	1632

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APPEAL BRIEF

Pursuant to 37 C.F.R. § 1.192, Appellants file this Appeal Brief in triplicate and respectfully request the Board to reconsider and reverse the Examiner's rejection in the above-identified application.

1. Real Party in Interest

Pursuant to an Assignment executed by each of the inventors of this application, the real party in interest is Arch Development Corporation. The Assignment was recorded on August 1, 2000.

2. Related Appeals and Interferences

None.

3. Status of the Claims

Claims 24-30, 32, 33, and 35-46 are pending and stand rejected. These are the claims being appealed. The remaining claims were cancelled (Claims 1-23, 31 and 34).

4. Status of Amendments

An Amendment under 37 C.F.R. §1.116, filed on March 24, 2003, was considered and entered by the Examiner, but failed to place the application in condition for allowance, as indicated in the Advisory Action dated April 9, 2003.

5. Summary of Invention

The present invention relates to adeno-associated virus (AAV) vector-mediated delivery of genes to transduce cardiac muscle cells, i.e., cardiomyocytes, perfused through a coronary artery or sinus. Specification, Page 4, Lines 12-16. More particularly, the presently claimed invention is directed to a method of introducing a nucleic acid encoding a desired molecule by infusing a recombinant adeno-associated virus (rAAV) vector into a coronary artery or a coronary sinus for a time and in particular amounts (i.e., about 10^5-10^9 infectious units (IU) AAV per gram body weight) sufficient to deliver the nucleic acid to cardiomyocytes that are perfused through the coronary artery or coronary sinus and thus to transduce the cardiomyocytes. The rAAV vector contains a transgene or nucleic acid

which encodes a desired molecule, such as an angiogenesis factor or an antisense RNA, and which is under the control of a control region, e.g., a promoter, operable in a cardiomyocyte. Upon expression of the nucleic acid in the cardiomyocyte, the transduced cardiomyocyte produces the desired molecule. Support for this specific subject matter is found in the Specification at Page 5, Lines 3-31 to Page 6, Lines, 1-12; Page 7, lines 24-30 to Page 8, Lines 1-2 and Lines 7-19; and Page 9, Lines 1-7.

6. Issues Presented

Whether Claims 24-30, 32, 33 and 35-46 are enabled in accordance with 35 U.S.C. § 112, first paragraph, with respect to making and using the claimed invention, and more particularly,

(a) whether the Examiner has provided a reasoned explanation why the evidence of record, including an expert declaration and post-filing evidence of success, fails to establish enablement of the claimed invention; and

(b) whether the specification would have taught one skilled in the art at the time of the invention, *i.e.*, in December 1998, that delivery of an rAAV vector as claimed to stably and efficiently transduce cardiomyocytes perfused through a coronary artery or a coronary sinus (Claims 24-30, 32, 33 and 35-46) could be achieved without undue experimentation.

7. Grouping of Claims

Claims 24-30, 32, 33 and 35-46 stand or fall together.

8. Argument

A. Introduction

Appellants respectfully submit that the claimed invention is enabled as evidenced by the declaratory evidence and post-filing successes of record. According to the Examiner, all of the claims were rejected under 35 U.S.C. § 112, first paragraph, for allegedly failing to "provide adequate guidance and evidence for the correlation of a desired molecule encoded by various nucleic acid [*sic*] with a particular cardiovascular disease or condition." Office Action, dated September 24, 2002, Page 3, first paragraph. The Examiner further opined that "[a]method of infusing a recombinant rAAV vector expressing a desired molecule into a coronary artery or a coronary sinus so as to stably and efficiently transduce cardiomyocytes perfused through said artery or said sinus does not have specific and substantial utility", in the context of the § 112, first paragraph, rejection. Office Action, dated September 24, 2002, Pages 3-4.

In response to the September 24, 2002 rejection, Appellants provided numerous uses for their demonstrated method of stable and efficient gene transfer into cardiomyocytes, namely, (1) delivery of marker genes to study gene expression in the heart; (2) delivery of therapeutic genes to treat acquired and inherited cardiac conditions; and (3) delivery of disease causing genes to create organ and animal models useful in developing therapeutic drugs for those specific diseases. Amendment, March 24, 2003, Page 6, first paragraph.

For marker gene expression studies, the marker genes can be coupled to promoters, or promoter fragments, to assess promoter activity based on the level of marker gene expression. With the appropriate promoters controlling expression of a marker gene, Appellants submitted that the presently claimed method can be used to evaluate the regulation and expression of genes in the functioning heart, including hearts in particular disease states, and provided by way of support a publication of Aikawa et al. (2002, *J. Biol. Chem.*, 277:18979-18985) demonstrating the use of rAAV to evaluate the cardiac muscle-specific alpha myosin heavy chain promoter. Appellants further provided the publication of Kawada *et al.* (2002, *Proc. Natl. Acad. Sci. USA*, 99:901-906) evidencing the well-established use of providing a marker gene as a control for experiments in which therapeutic genes are provided. Kawada et al. used rAAV encoding lacZ (aka β-galactosidase) as a control for somatic gene therapy for dilated cardiomyopathy (DCM) in the TO-2 hamster strain receiving rAAV encoding the δ-sarcoglycan (δ-SG) and demonstrated delivery of a therapeutically-effective amount of a gene product to treat a cardiac condition using Applicants claimed method of intracoronary delivery.

In addition, Appellants submitted the Declaration of Dr. Michael Parmacek, a renowned scientist in the area of cardiovascular gene therapy, who declared that at the time of the claimed invention, those having skill in the art would recognize further utilities for the claimed invention, including uses to deliver genes to establish organ models and animal models for human cardiovascular disease. Parmacek Declaration,

¶ 22.

Dr. Parmacek also positively stated that at the time of this invention there was sufficient knowledge and skill in the art of gene therapy, which together with the information and guidance provided in Appellants' specification, are sufficient to enable the skilled artisan to make and use the claimed invention. The specification thus teaches the skilled artisan how to construct an rAAV vector encoding a molecule of interest, i.e., a protein with therapeutic potential, for example, an angiogenesis factor or an antisense RNA, and to infuse the vector into the blood flow of a coronary artery or coronary sinus of an animal with an expectation that the results would be successful viral vector transduction of cardiomyocytes perfused through the coronary artery or sinus and expression of the molecule of interest. Parmacek Declaration, ¶¶ 10, 14-19 and 24-27. Dr. Parmacek's opinion relied on following facts known at the time of filing:

- Construction of an rAAV vector encoding a desired molecule operably linked to a control region would have been routine;
- Many proteins and antisense RNA sequences useful for treating cardiovascular conditions were known, and cloned DNA encoding these proteins and RNA sequences was readily available;
- Transduction of mouse or rat cardiomyocytes *in vitro* or *ex vivo* using rAAV vectors encoding a marker gene, i.e., β-galactosidase, led to detectable expression of the marker gene;
- *In vivo* intracardiac injection of rAAV encoding the marker gene β-

galactosidase in rats resulted in the expression of β-galactosidase in myocardial cells for up to 2 months post injection;

- Therapeutic effectiveness of *in vivo* rAAV vector transduction was demonstrated in rodent cells. Specifically, intraventricular or intracardiac injection of rAAV encoding angiotensinogen receptor (AT_1-R) antisense RNA reduced blood pressure and slowed the development of hypertension in rats;
- rAAV vectors were used *in vivo* to express genes encoding various markers and therapeutic proteins, such as alkaline phosphatase, amino acid decarboxylase, human Factor IX, and cystic fibrosis transmembrane conductance regulator, in the organs of animals other than mice or rats; and
- rAAV vectors were particularly suited for use as gene transfer vectors given their (i) stable integration into the host cell's genome, (ii) long-term transgene expression, (iii) lack of immunogenicity, and (iv) ability to transduce non-dividing cells.

In light of the above, Dr. Parmacek concluded that the claimed invention could be practiced without undue experimentation at the time of filing.

Further, Dr. Parmacek reviewed a post-filing date publication and positively declared that others in the pertinent art merely followed Appellants' disclosed methods and successfully transduced mouse cardiomyocytes using rAAV vectors encoding the marker gene β-galactosidase under the control of the cytomegalovirus (CMV) promoter. Parmacek Declaration, ¶ 30. To summarize, in Dr. Parmacek's stated opinion, the

claimed invention required no significant or undue experimentation and was enabled at the time of filing.

After reviewing the evidence, the Examiner issued a Final Rejection and an Advisory Action that in substance did no more than acknowledge Appellants' evidence, and maintained that *in vivo* gene therapy was not well developed and unpredictable at the time of the invention (Advisory Action, Page 3, First paragraph), and that Appellants had failed to provide sufficient description for the use of the claimed method in evaluating the regulation and expression of any gene in a functioning heart in particular disease states. The Examiner found Dr. Parmacek's declaration to be unpersuasive because the specification was considered not to "provide adequate guidance and evidence for what gene can be delivered to establish what type of organ or animal model for what human cardiovascular disease." Advisory Action, Page 2, Last paragraph. The Examiner's response is conclusory and improper: it lacks factual findings that suggest that the specifically claimed subject matter is not enabled, and it does not address the merits of the evidence. Thus, the rejection and the Advisory Action lack an adequate factual explanation of why the evidence of record did not overcome the rejection.

The claimed infusion method to introduce a nucleic acid encoding a desired molecule, e.g., a protein or an antisense RNA, into cardiomyocytes using particular concentrations of rAAV vector is enabled with respect to delivery of a marker gene. Specifically, Appellants demonstrated that rAAV encoding lacZ transduces 40-50% of cardiomyocytes when about 10^9 IU were delivered by infusion into a coronary artery of a

mouse heart. Specification, Example, pages 10-11. Numerous uses of Appellants' method are clear to those having skill in the pertinent art and have been elucidated above.

At the time of the invention, rAAV vectors encoding markers and therapeutic proteins, and administered by non-infusion routes known to transduce rodent cells, resulted in the expression of therapeutically effective amounts of several genes in various organs of non-rodent animals. Construction of an rAAV vector encoding a desired molecule, delivery to an animal by infusion into a coronary artery or a coronary sinus, and transduction of cardiomyocytes according to Appellants' claimed method do not require undue experimentation (either then or now), a conclusion concurred with in the expert declaration of Dr. Michael Parmacek. Finally, post-filing evidence of record demonstrates that by following the teaching of the specification, and nothing more, at least one different group has demonstrated that the invention works as claimed.

Accordingly, Appellants submit that the specification teaches those of skill in the art to make and use the presently claimed invention without the practice of undue experimentation.

B. The Law of Enablement

The law is well established that to satisfy the enablement requirement, the specification must teach those of skill in the art how to make and use the claimed subject matter at the time of filing without resort to undue experimentation. *In re Wands*, 858 F.2d 731, 737, 8 USPQ.2d 1400, 1404 (Fed. Cir. 1988); *In re Wright*, 999 F.2d 1557,

1562, 27 USPQ.2d 1510, 1513 (Fed. Cir. 1993); *Johns Hopkins University v. CellPro, Inc.*, 152 F.3d 1342, 1354, 47 USPQ.2d 1705 (Fed. Cir. 1998). In this regard, the PTO has the initial burden to set forth a reasonable explanation or provide evidence showing why the description provided would not enable the scope of the claimed invention. Once this burden is met, then the burden shifts to the Appellant to provide suitable evidence that the specification is indeed enabling. *In re Wright,* 999 F.2d at 1561-1562.

A specification which contains a teaching of how to make and use claimed subject matter **must** be taken as enabling unless there is reason to doubt the objective truth of the statements relied upon in the specification. *In re Brana*, 51 F.3d 1560, 1566, 34 USPQ.2d 1436 (Fed. Cir. 1995) (emphasis in original; citing *In re Marzocchi*, 439 F.2d 220, 223, 169 USPQ 367, 369). Furthermore, the enablement standard is an objective standard. It is irrelevant whether the teaching in the specification is accomplished by broad terminology or illustrative examples. *In re Wright,* 999 F.2d at 1561. In fact, there is no requirement that a working example be provided. *In re Strahilevitz*, 668 F.2d 1229, 1232, 212 USPQ 561 (CCPA 1982). The dispositive issue is whether, in light of the disclosure and the level of ordinary skill in the art, the invention could have been made and used without undue experimentation at the time of filing. *Id.* at 1232.

To determine whether experimentation is undue requires a consideration and balancing of the individual facts of each case. *In re Wands,* 858 F.2d at 737-740, 8 USPQ.2d at 1404-1407. The eight "Wands" factors, discussed in detail below, are widely relied upon to make this determination. However, other factors can be considered and it

must be remembered that no single factor is determinative nor does any single factor necessarily deserve more weight than any other. *Id.* at 737, 1404.

Finally, while enablement is viewed as of the time of filing, post-filing events are relevant to enablement when they show the state of the art at the time of filing or prove the invention works as broadly as claimed. *Gould v. Quigg*, 822 F.2d 1074, 1078, 3 USPQ2d 1302, 1305 (Fed. Cir. 1987). *See also Johns Hopkins Univ. v. CellPro, Inc.*, 931 F. Supp. 303, 323 (D. Del. 1996), *aff'd in relevant part*, 152 F.3d 1342, 47 USPQ2d 1705 (Fed. Cir. 1998).

C. No Rebuttal Evidence

The initial burden to set forth a reasonable explanation or provide evidence showing why the specification does not enable the scope of the claimed invention resides with the PTO. As stated in *In re Marzocchi*, 439 F.2d 220, 169 USPQ 367 (CCPA 1971), "it is incumbent upon the Patent Office, whenever a rejection on this basis is made, to explain why it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement. Otherwise, there would be no need for the applicant to go to the trouble and expense of supporting his presumptively accurate disclosure." 169 USPQ at 370. MPEP 2164.04.

Appellants respectfully submit that the Examiner's burden has not been and cannot be met simply by expressing skepticism that one of ordinary skill in the art would

not believe that the invention works because the state of the prior art of gene therapy *in vivo* was not well developed and was unpredictable at the time of the invention. Appellants provided a sufficient showing that an rAAV vector encoding lacZ transduced 40-50% of cardiomyocytes when 10^9 IU of the vector was infused *in vivo* into a coronary artery of a mouse heart. Appellants have demonstrated that the invention works as claimed. Furthermore, unpredictability of the gene therapy field as a whole is an inappropriate grounds upon which to reject the present invention, particularly since it is drawn to specific subject matter (discussed below in Section D). The question of unpredictability must be applied to the terms of the claimed subject matter -- not to unfounded and unsubstantiated generalizations regarding the claimed subject matter and its use.

The Examiner makes conclusory statements that the specification "fails to provide adequate guidance and evidence for the correlation of a desired molecule encoded by various nucleic acid [*sic*] with a particular cardiovascular disease or condition". Office Action, dated September 24, 2002, Page 3; Advisory Action, dated April 9, 2003, Page 3. However, the instant specification teaches a number of different molecules whose encoding nucleic acids can be encoded by the rAAV vectors of the invention. In particular, various proteins that can be administered to treat or ameliorate various cardiovascular conditions are disclosed on Page 5, Lines 12-31 of the Specification; antisense RNA is another suitable molecule for use in the claimed method. Specification, Page 6, Lines 5-12.

Furthermore the Examiner's additional statements that the specification fails to provide adequate guidance and evidence "whether the desired molecule would be expressed and be present in a sufficient amount at the targeted site such that the desired molecule could provide therapeutic effect for a particular cardiovascular disease or condition in a patient *in vivo*" and that "gene therapy *in vivo* using a rAAV vector expressing various gene product, such as antisense RNA, contractile protein, a growth factor, an angiogenic factor, a FGF, a VEGF etc., to treat cardiovascular diseases needs to be considered separately because different gene products has [*sic*] different biological functions and pathologies of different cardiovascular diseases differ, and the unpredictable nature of gene therapy *in vivo.*" [*sic*] (Advisory Action dated April 9, 2003 at Page 3) belie the statements of Dr. Parmacek concerning expression of genes transduced by rAAV, as well as their expression and therapeutic efficacy. Parmacek Declaration, ¶¶ 24-26.

Rather, the Examiner is required to provide reasoning or evidence that specifically addresses the elements of the claimed subject matter. This has not been done. For example, the Examiner has not provided any evidence or reason to doubt that, as Dr. Parmacek has declared, the skilled practitioner in December 1998 could have carried out the claimed invention without undue experimentation, and that no further information beyond the teachings of the specification and the prior art is needed to practice the claimed invention.

Further, the Examiner has stated that "[d]oses, schedules, responsive variables, required level and stability of gene expression, and criteria of success all depend on the gene used and the particular cardiovascular disease targeted." However, the record does not provide any credible evidence or sufficiently reasoned explanations upon which to base this assertion. For example, the instant Specification teaches when efficient transduction occurs (Specification, Page 8, Lines 20-26), time of infusion (Specification, Page 8, Lines 27-30), and amounts of rAAV suitable for infusion (Specification, Page 9, Lines 1-7). Dr. Parmacek also opined that one having skill in the art would be able to practice the invention as claimed based only on the teachings of the specification and knowledge possessed by the practitioner in the art. Parmacek Declaration, ¶¶ 27 and 29. The Examiner makes several general assertions, yet does not relate how these assertions have any bearing subject matter as claimed.

Thus, for at least the foregoing reasons, Appellants submit that the rejection of the pending claims for lack of enablement is improper as a matter of law and should be withdrawn.

D. Undue Experimentation

As previously stated, the issues center on whether the specification taught one skilled in the art at the time of the invention, *i.e.*, in December 1998, that delivery of an rAAV vector encoding a desired molecule stably and efficiently transduces cardiomyocytes perfused through a coronary artery or a coronary sinus could be achieved

by the claimed method without undue experimentation. The Wands case provides eight factors to be considered and weighed together, with or with out additional factors, to establish whether or not the claimed invention is so enabled. The eight Wands factors are (1) breadth of claims, (2) nature of the invention, (3) state of the art, (4) level of skill in the art, (5) level of predictability, (6) amount of direction and guidance in the specification, (7) presence or absence of working examples, and (8) quantity of experimentation. Each factor is evaluated below in light of the evidence and other information of record or included herewith, including the statements of Dr. Parmacek.

1. Breadth of claims

The breadth of the claims refers to the scope of coverage that the claims are afforded. The scope of enablement provided by the disclosure in view of the state of the art must be commensurate with the scope of protection being sought.

The claims are drawn to a single route of administration, namely infusion of a specified amount of an rAAV vector encoding a desired molecule into a coronary artery or coronary sinus of an animal to transduce cardiomyocytes that are perfused through the coronary artery or coronary sinus. The specification provides direct data showing that gene transfer by this mode of delivery permits transduction and expression of genes in cardiac muscle cells using an rAAV vector.

The claims are drawn to a single class of gene transfer vector, namely an rAAV vector. The data in the specification unequivocally demonstrate to the skilled artisan that

an rAAV virus vector carrying a nucleic acid (or "transgene") encoding a given protein or molecule stably transduces cardiomyocytes, and further that the transgene (e.g., β-galactosidase) harbored by such a vector is expressed. Specification, Example, Pages 10-11; and Parmacek Declaration, ¶ 10.

The specification teaches that transgenes that can be encoded by an rAAV vector (Specification, Pages 5-6) and the Parmacek declaration asserts that various nucleic acids encoding molecules for treating and ameliorating cardiac conditions were known and available in the art, and that genes could be transduced into and expressed by cardiomyocytes in rodents and other non-rodent animals using rAAV vectors. Parmacek Declaration, ¶¶ 11, 12, 14, 17, 18, 20 and 24-26. Hence, by reciting that a specified amount of rAAV vector encoding a desired molecule is transduced into cardiomyocytes perfused through coronary artery or coronary sinus, Appellants' claims, when considered in light of the specification, are reasonably correlated to a method demonstrated to be feasible with the claimed vector encoding a nucleic acid molecule as taught in the specification and known and available in the art at the time of the invention. Furthermore, Appellants' method is appropriately tailored to achieve the claimed result, which is to transduce cardiomyocytes with the vector DNA after infusing the vector into a coronary artery or sinus through which the target cardiomyocytes are perfused.

The claims recite that the rAAV contains at least one nucleic acid that encodes a desired molecule and that the nucleic acid is operably linked to a control region. In this regard, the claims are rightly broad as it was a matter of routine skill for the practitioner in

the art to select any of numerous and known nucleic acids, as well as strong promoters or tissue-specific promoters to express a desired nucleic acid in a desired tissue. Parmacek Declaration, ¶¶ 11, 12, 13 and 23.

With respect to the breadth of the claims and based on the reasoning and evidence provided above, Appellants submit that the present claims are commensurate in scope with the teaching, disclosure and guidance provided in the Specification. Thus, Appellants believe the scope of the claims is reasonable and in accordance with the teaching of the specification.

2. Nature of the Invention

The nature of the invention is important for determining the state of the art and the level of skill in the art. The claimed subject matter relates to an *in vivo* method of gene transfer to transduce cardiomyocytes with a virus vector, namely, rAAV. The method involves a specific mode of delivery, i.e., infusion, to transduce cardiac muscle cells with specified amounts of rAAV containing at least one nucleic acid encoding a desired product, so that the product encoded by the nucleic acid is stably expressed in the cardiac muscle cells. This process of coronary artery or coronary sinus perfusion leads to the stable transduction of cardiomyocytes throughout the myocardium. This area of cardiovascular gene transfer is a highly specialized subfield of genetic approaches to treating cardiac-related disease conditions, and is a large multi-faceted discipline covering gene transfer of nucleic acids, viral vectors and recombinantly-transduced cells for

somatic gene therapy and for the generation of immune responses. By December 1998, somatic gene therapy was well beyond its infancy, for example, the first human clinical trials began in September 1990, and by February 1992, thirteen different human gene therapy clinical trials had been approved by the Recombinant DNA Advisory Committee (RAC) of the NIH and many of these by the FDA. Miller, 1992, *Nature*, 357:455-460 (June 11, 1992 issue).

3. State of the art

The state of the art is what one of skill in the art would have known at the time of filing. It establishes the relevant field of endeavor, *i.e.*, the pertinent art, what was well known in the art, the degree of predictability in the art, and the amount of guidance that the specification needs to be enabling. Such guidance must always be considered with that which is well known, since the specification need not disclose, and preferably omits, that which is well known. *In re Buchner*, 929 F.2d 660, 661, 18 USPQ.2d 1331, 1332 (Fed. Cir. 1991).

The claimed invention must be viewed in conjunction with the information available to the skilled artisan in December 1998. At that time there was already a significant and growing body of knowledge relating to gene therapy. In fact, the specification discusses the state of the art.

Before December 1998, it had been demonstrated that the *in vivo* intracardiac injection of rAAV encoding the β-galactosidase marker gene in rats resulted in the

expression of β-galactosidase in myocardial cells for up to 2 months post-injection. Kaplitt *et al.* (1996), *Ann. Thorac. Surg.* 62:1669-1676. Parmacek Declaration, ¶ 16.

Before December 1998, several groups had demonstrated the therapeutic effectiveness of *in vivo* rAAV vector transduction in rodent cells. The intraventricular or intracardiac injection of rAAV encoding angiotensinogen receptor (AT_1-R) antisense RNA reduced blood pressure and slowed the development of hypertension in rats. Phillips *et al.* (1997), *Hypertension* 29:374-380. Therapeutic concentrations of the gene encoding Factor IX had been expressed in mouse hepatocytes using rAAV vectors infused into the portal vein. Snyder *et al.* (1997), *Nature Genet.* 16:270-276. Therefore, one of skill in the art would have understood that successful transduction with therapeutic molecules would have therapeutic benefit. Parmacek Declaration, ¶ 17.

Before December 1998, rAAV vectors had been used *in vivo* to express genes encoding various markers and therapeutic proteins in the organs of animals other than mice or rats. For example, the alkaline phosphatase (AP) reporter gene was expressed in the carotid adventitia of cynomolgus monkeys using rAAV vectors infused or injected into the carotid artery. Lynch *et al.* (1997) Circ. Res. 80:497-505. Another study demonstrated sustained expression of human Factor IX in hemophilic dogs following intramuscular injection of rAAV vectors encoding the clotting factor. Monahan *et al.* (1998), *Gene Ther.* 5:40-49. Further, the gene encoding amino acid decarboxylase (AADC) had been expressed at levels sufficient to partially correct dopamine deficiencies in the caudate of green monkeys using injected rAAV vectors. During *et al.* (1998), *Gene*

Ther. 5:820-827. Finally, the gene encoding cystic fibrosis transmembrane conductance regulator (CFTR) had been expressed in the bronchial epithelium of rabbits and rhesus monkeys using aspirated rAAV vectors. Flotte *et al.* (1993), *Proc. Natl. Acad. Sci. USA* 90:10613-10617 and Conrad *et al.* (1996), *Gene Ther.* 3:658-668, respectively. The levels of CFTR RNA expressed in the rhesus monkeys were similar to endogenous levels in humans. Conrad *et al.* (1996), *Gene Ther.* 3:658-668. Parmacek Declaration, ¶ 18.

As further declared by Dr. Parmacek, a Phase I human trial of rAAV-CFTR gene vectors in human cystic fibrosis subjects was initiated in August 1999, based at least in part on several of the studies mentioned above. Flotte *et al.* (1996), *Hum. Gene Ther.* 7:1145-1159 (particularly Section 3.4) and "Phase I Randomized Study of Adeno-Associated Virus-CFTR Vector in Patients with Cystic Fibrosis," www.clinicaltrials.gov/ct/gui/c/w1r/show/NCT00004533?order=1&JservSessionIdzone_ct=xnrwsoycu1 (downloaded from website on June 13, 2002). Parmacek Declaration, ¶ 19.

In December 1998, it was well known that rAAVs were particularly suited for use as gene transfer vectors given their stable integration into the host cell's genome, long-term transgene expression, lack of immunogenicity, and ability to transduce non-dividing cells. Flotte *et al.* (1996), *Hum. Gene Ther.* 7:1145-1159 (particularly Section 3.2); Gnatenko *et al.* (1997), *J. Invest. Med.* 45:87-98 (particularly p. 88, col. 1, first full paragraph); Maeda *et al.* (1997), *Cardio. Res.* 35:514-521 (particularly pp. 514-515, bridging paragraph); Phillips *et al.* (1997), *Hypertension* 29:374-380 (particularly p. 379, col. 2, first full paragraph). Parmacek Declaration, ¶ 20.

As of December 1998, the necessary techniques and materials were available in the art to construct a rAAV vector encoding a molecule of interest operably linked to a control region. Moreover, as of this time, many proteins and antisense RNA sequences useful for treating cardiovascular conditions were known, and cloned DNA encoding these proteins and RNA sequences was readily available. For example, cloned DNA encoding many angiogenic factors, including acidic FGF, basic FGF, FGF-5, platelet-derived growth factor (PDGF), angiogenin, and vascular endothelial growth factor (VEGF), was available. Connolly *et al.* (1991), *J. Cell. Biochem.* 47:219-223; Crumley *et al.* (1990), *Biochem. Biophys. Res. Commun.* 171:7-13; Folkman *et al.* (1987), *Science* 235:442-447; Kurachi *et al.* (1985), *Biochemistry* 24:5494-5499; Kurokawa *et al.* (1987), *FEBS Lett.* 213:189-194; Leung *et al.* (1989), *Science* 246:1306-1309; Schaper *et al.* (1991), *Basic Res. Cardiol.* 86(Suppl. 2):51-56. Hence, it would have been routine to make the appropriate vector at the time of the invention. Parmacek Declaration, ¶¶ 11 and 12.

In December 1998, it was routine to prepare titers of AAV vectors and to determine doses of AAV vectors to transduce cells, e.g., cardiomyocytes, human kidney cells and fibroblasts. This constitutes background information relating to the state of the art at the time of Appellants' invention.

However, it is the Appellants who provide to the art the amounts of rAAV vector that are needed to achieve high efficiency, stable transduction of cardiac muscle cells according to the claimed method. The amounts of rAAV needed to attain high efficiency

and stable transduction of cardiomyocytes with rAAV were neither routine nor part of the state of the art at the time of Appellants' claimed invention. Only the specification of the instant application provides the teaching of such doses. Specifically, Appellants' specification teaches that a preferred amount of infused AAV vector can range from about 10^5 infectious units (IU) AAV per gram body weight of the subject animal to about 10^9 IU AAV per gram body weight, preferably from about 10^6 IU AAV per gram body weight to about 10^8 IU AAV per gram body weight, and most preferably about 5×10^7 IU AAV per gram body weight to about 6×10^7 IU AAV per gram body weight. In the Example in the specification, a mouse heart is perfused with 1.5×10^9 IU AAV per gram body weight. *See also,* Parmacek Declaration, ¶ 13.

4. Level of skill in the art

At the time of the invention, the level of skill in the art of gene transfer and expression into the cardiovascular system was high. For example, in December 1998, researchers in this scientific field would have had a medical degree (M.D.) and/or a doctorate degree (Ph.D.) in an area such as cardiology, anatomy, molecular biology, or in a related field such as physiology, and at least several years research and/or clinical experience following completion of the advanced degree. In addition, those of skill in the art would also have had at least one or more years of actual experience in the field of gene transfer. Parmacek Declaration, ¶ 21. In fact, Dr. Parmacek was an active researcher in

the cardiovascular gene therapy field well prior to December 1998, and qualifies as one of skill in the art at the time of the invention.

5. Level of predictability

Predictability is the ability to extrapolate from disclosed or known results to the untested. One means to measure predictability is derived from the amount of guidance needed for enablement; the less guidance needed, the greater the predictability. *In re Fisher*, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1971).

Appellants consider there to be a reasonable level of predictability in the art of gene transfer and gene therapy in December 1998, because the skill level in the art was high, many molecules for the treatment and amelioration of cardiac conditions and diseases were known (at least three had been shown stable enough to exert biological activity *in vivo*), protein expression was obtainable from marker genes and therapeutic enzymes, and the state of the art was advancing rapidly.

6. Amount of direction and guidance in the application

The specification provides ample direction and guidance to make and use the present invention as discussed in detail by Dr. Parmacek. Parmacek Declaration, ¶¶ 27 and 29.

The specification teaches AAV vectors for use in the invention and discloses that such vectors were well known in the art and could be prepared by standard

methodologies. At least two references are cited as sources of that information. Specification, Page 4, Lines 24-31 to Page 5, Lines 1-2.

The specification states that methods to make, use and propagate AAV vectors containing transgenes are known in the art. Specification, Page 4, Lines 30-31 to Page 5, Lines 1-2. The specification teaches nucleic acid sequences of interest or "transgenes" which code for any product. Specification, Pages 5-6. The coding sequences for hundreds of gene products were known well before December 1998 (i.e., as early as November 1992), as well as the various "landmarks" to identify the relevant transcription, translation and processing signals and open reading frames. There is nothing unusual in this regard for genes encoding molecules that can treat or ameliorate cardiovascular conditions, such as molecules that are competent to induce angiogenesis.

The specification more specifically identifies molecules that can be transduced into cardiomyocytes, particularly those which treat or ameliorate a number of cardiac conditions. Specification, Page 5, Lines 12-31 to Page 6, Lines 1-12.

The specification identifies regulatory elements, including suitable promoters and enhancers for use in the rAAV vectors; promoters may be tissue-specific, e.g., for cardiomyocytes, or they may be promiscuous. Specification, Page 6, Lines 25-30 to Page 7, Lines 1-2.

The specification teaches how to perform delivery of rAAV vectors by infusion into a coronary artery or coronary sinus of an animal to transduce cardiomyocytes perfused through the artery or sinus, and demonstrates that such delivery of an rAAV

vector encoding a β-galactosidase marker gene resulted in expression of that gene in cardiomyocytes throughout the myocardium as evidenced by the observation of X-gal staining in the cardiomyocytes. Specification, Example, Pages10-11.

Given the high level of skill in the art, such guidance and direction is sufficient for those of skill in the art. Indeed, others in the field have done nothing more than follow the teaching of the specification to successfully transduce mouse cardiomyocytes with a marker gene in accordance with the claimed methods. Parmacek Declaration, ¶ 28.

7. Presence or absence of working examples

The specification demonstrates a working example of the claimed delivery method showing infusion of rAAV encoding a marker gene into the blood flow of a coronary artery. Specification, Example, Page 11. From this example, the skilled artisan at the time would conclude that infusion of an rAAV vector into the coronary artery leads to transduction of cardiac muscle cells and expression of the gene encoded therein as described in the specification and as discussed in detail by Dr. Parmacek. Parmacek Declaration, ¶¶ 10, 24 and 27.

8. Quantity of experimentation

The quantity of experimentation refers to that amount of experimentation that is needed to be performed by one skilled in the art to make and use the invention. In this regard, Appellants note that the cost and time needed to perform any experimentation are not controlling factors, rather the test is whether the experimentation is merely routine and

whether there is sufficient guidance with respect to any experimentation that should be needed. In the present case, any needed experimentation, if even considered as such in 1998, is merely routine.

All of the materials needed to practice the invention are available in the art. At the time of the invention, all of the techniques needed to construct an rAAV vector encoding a desired molecule under control of regulatory elements operable in cardiac muscle cells were known. This information has already been established on the record in the responses to the previous Office Actions. Moreover, Dr. Parmacek concurs. Parmacek Declaration, ¶¶ 11, 12 and 23.

With the desired construct in hand, the next step is to prepare an animal for delivery of the vector and to deliver that vector by the claimed method of the invention. Such information is provided in the working Example of the specification with respect to a mouse. The techniques applicable for the mouse are generally applicable for all experimental animals and humans, and any variations needed could be readily determined without undue experimentation. For example, experimental animal models for cardiovascular studies were readily available at the time invention for such animals as mice, rats, pigs and dogs. The procedure would thus follow the working Example of the specification using the newly constructed vector.

After administration of the vector to a number of animals sufficient to provide a statistically significant result (and appropriate controls), the skilled practitioner could determine if cardiomyocytes had been transduced and expressed the encoded molecule.

The techniques for such determinations were known in the art at the time of the invention; examples of assessing enzyme activity and staining for a transduced enzyme were provided in the specification. The preferred initial starting point for these experiments would be to use the amount of rAAV vector from the Example (i.e., 1.5×10^9 IU) encoding the molecule of interest, e.g., β-galactosidase. It would be routine thereafter to vary AAV vector dosages to determine if cardiomyocytes are transduced. Parmacek Declaration, ¶¶ 13 and 27.

9. Post Filing Evidence

Dr. Parmacek has reviewed a filing reference which is of record, namely, Svensson *et al.* (1999), *Circulation* 99:201-205. Dr. Parmacek concurs that the results of the reference merely followed the teachings provided in Appellants' specification (and as claimed) to transduce cardiomyocytes using rAAV vectors encoding the marker gene β-galactosidase under the control of the CMV promoter. Parmacek Declaration, ¶ 28. Likewise, the reported study used rAAV vectors and promoters that were well known before the time that Appellants filed the instant application.

D. Conclusion

In Dr. Parmacek's opinion, the specification and the state of the art enabled one of skill in the art to practice the claimed invention in December 1998. Parmacek Declaration, ¶¶ 23-29. Based on this analysis, it is clear that:

- In December 1998, it would have been a matter of routine experimentation to

construct a recombinant AAV vector encoding a desired molecule operably linked to a control region for the transduction of cardiomyocytes. The mechanics of selecting vectors, genes, and promoters and combining them into expression constructs were well known at that time. Moreover, cloned DNA encoding proteins and antisense RNA useful for treating cardiovascular conditions was available for incorporation into these expression constructs. Hence, one of skill in the art at the time of the invention could have selected a combination of rAAV vectors, regulatory elements, such as promoters and genes suitable to produce the expression of a desired molecule in cardiomyocytes. Parmacek Declaration, ¶ 23.

- At the time of the invention, effective transduction of various rodent and human cells using rAAV vectors encoding marker and therapeutic genes had been demonstrated. Parmacek Declaration, ¶ 24.
- Genes encoding markers and therapeutic proteins had been expressed in various organs of animals other than rodents using rAAV vectors administered by some of the same routes known for transduction of rodent cells. For example, canine and primate cells expressed therapeutic genes, Factor IX and amino acid decarboxylase (AADC), respectively, following local injection of rAAV vectors. Parmacek Declaration, ¶ 25.
- At the time of the invention, rAAV vectors had been used to express therapeutically-effective amounts of several genes in the liver, brain,

vasculature, and lungs of various non-human animals. A phase I trial to determine the therapeutic effectiveness of rAAV-CFTR gene vectors in human subjects had also been initiated. Parmacek Declaration, ¶ 26.

- The specification of the application and the state of the art enabled one of skill in the art to practice the claimed invention in December 1998. Based on such teaching and guidance in Appellants' specification, along with the state of the art described herein with respect to molecular biological techniques and *in vivo* gene transfer, it was merely a matter of routine experimentation to construct a recombinant AAV vector encoding a desired molecule, deliver it to an animal by infusion into a coronary artery or coronary sinus, and thereby transduce cardiomyocytes perfused through the coronary artery or coronary sinus. Parmacek Declaration, ¶ 27.
- Since the filing of the instant specification, others in the field have done nothing more than follow the teaching of the specification and have successfully transduced mouse cardiomyocytes using rAAV vectors encoding the marker gene β-galactosidase under the control of the CMV promoter. Svensson *et al.* (1999), *Circulation* 99:201-205. Parmacek Declaration, ¶ 28.

In conclusion and based on the foregoing, the specification together with the state of art at the time, provided one of skill in the art with sufficient guidance to make and use

the full scope of the claimed invention without requiring undue experimentation. Parmacek Declaration, ¶ 29.

In view of the above, Appellants respectfully request that the Board of Patent Appeals and Interferences reverse the outstanding rejections and remand the Application to the Examiner to enter a Notice of Allowance.

Respectfully submitted,

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Appendix

Pending Claims of U.S. Serial No. 08/473,830

24. A method of introducing a nucleic acid encoding a desired molecule into cardiomyocytes which comprises:

infusing a recombinant adeno-associated virus (AAV) vector into a coronary artery or a coronary sinus of an animal in an amount of about 1 x 10^5 to about 1 x 10^9 infectious units (IU) AAV per gram body weight and for a time sufficient to stably and efficiently transduce cardiomyocytes perfused through said artery or said sinus, wherein said AAV vector comprises at least one nucleic acid operably linked to a control region, said nucleic acid encoding said desired molecule.

25. The method of claim 24, wherein said AAV transduces at least about 10% of said cardiomyocytes.

26. The method of claim 24, wherein said AAV transduces at least about 40% of said cardiomyocytes.

27. The method of claim 24, wherein said AAV transduces at least about 50% of said cardiomyocytes.

28. The method of claim 24, wherein said AAV is infused for at least about 2 minutes to about 30 minutes.

29. The method of claim 24, wherein said AAV is infused for at least about 5 minutes to about 20 minutes.

30. The method of claim 24, wherein said AAV is infused for about 15 minutes.

32. The method of claim 24, wherein said amount of AAV is about 1 x 10^6 IU AAV per gram body weight to about 1 x 10^8 IU AAV per gram body weight.

33. The method of claim 32, wherein said amount of AAV is about 6 x 10^7 IU AAV per gram body weight.

35. The method of claim 28, wherein about 1 x 10^6 IU AAV per gram body weight to about 1 x 10^8 IU AAV per gram body weight is infused.

36. The method of claim 35, wherein about 6 x 10^7 IU AAV per gram body weight is infused.

37. The method of any one of claims 28, 35 or 36, wherein said AAV is infused for about 5 to about 20 minutes.

38. The method of claim 37, wherein said AAV is infused for about 15 minutes.

39. The method of claim 24, wherein about 6 x 10^7 IU AAV per gram body weight is infused for about 15 minutes.

40. The method of claim 24, wherein said coronary artery is infused *ex vivo* or *in vivo*.

41. The method of claim 24, wherein said desired molecule is an anti-sense RNA or a protein.

42. The method of claim 24, wherein said desired molecule is an ion channel gene, a contractile protein, a phospholamban, a β adrenergic receptor, a β adrenergic kinase, a growth factor, an angiogenic factor, a protein or nucleic acid capable of inducing angiogenesis, or a protein or nucleic acid capable of inhibiting angiogenesis.

43. The method of claim 24, wherein said desired molecule is FGF-1, FGF-2, FGF-5, VEGF, or HIF-1.

44. The method of claim 24, wherein said desired molecule is thymidine kinase, p2l, p27, p53, Rb or NF-κB.

45. The method of claim 24, wherein said cardiomyocytes are in an individual having a vascular condition selected from the group consisting of restenosis, atherosclerosis, congestive heart failure, ischemic cardiomyopathy, malignant arrhythmia, myocardial infarction, congestive heart failure, and dilated and hypertrophic cardiomyopathy.

46. The method of claim 24, wherein said desired molecule has an effect selected from the group consisting of inducing angiogenesis, inhibiting angiogenesis, stimulating or inhibiting cell proliferation, treating restenosis, treating atherosclerosis, treating congestive heart failure, treating ischemic cardiomyopathy and treating malignant arrhythmia.